Regioselective oxidation of carbohydrates

Eisink, Niek Nicodemus Henricus Maria

Chapter 2

Regioselective Oxidation of Unprotected (1→4) Linked Glucans

In this chapter, the palladium-catalysed oxidation of unprotected (1→4) linked glucans is described. This chemo- and regioselective oxidation is demonstrated in the two-step bisfunctionalization of (1→4) linked glucans up to the 7-mer. Introduction of an anomeric azide is followed by a highly regioselective mono-oxidation of the terminal C3-OH functionality. The resulting orthogonal bis-functionalized oligosaccharides are a viable alternative to PEG-spacers as demonstrated in the conjugation of a cysteine mutant of 4-oxalocrotonate tautomerase with biotin.

This chapter is adapted from the original publication:
Eisink, N. N. H. M.; Lohse, J.; Witte, M. D.; Minnaard, A. J. *Org. Biomol. Chem.* **2016**, 14, 4859

2.1 Introduction

Complex molecule functionalization including their diversification is one of the frontiers of contemporary chemistry. Control over chemical reactivity and predictable selectivity are the key goals in this field. Important strategies that currently see considerable development are C-H bond oxidation[1] and C-H bond alkylation[2], given the large number of C-H bonds present in most organic molecules. The regioselective functionalization, and, in particular, oxidation, of oligosaccharides should be placed in the same ball park. In oligo- and polysaccharides, the number of hydroxyl groups roughly equals the number of C–H bonds. Whereas in C–H activation, control over chemical reactivity is challenging, in carbohydrate oxidation (regio)selectivity is the crux. Indeed, hardly any studies have appeared on this topic,[3,4] apart from those focusing on the anomeric hemiacetal, which stands out reactivity-wise.[5] Although based on literature the picture seems bleak, it is well-known that in monosaccharides acetal formation is often highly regioselective. This feature has been exploited to selectively oxidize hydroxyl groups via tin-acetals.[6] In the field of palladium-catalysed alcohol oxidation, Waymouth and coworkers have shown that 1,2-vicinal diols are selectively oxidized to hydroxyl ketones, e.g. the secondary hydroxyl group is oxidized preferentially over the primary hydroxyl group, via the palladium diol-complex.[7] We recently extended this approach by demonstrating that this catalyst also discriminates between multiple secondary hydroxyl groups and oxidizes mono- and diglucosides selectively at the C3 position.[8]

In this chapter, we have further extended the scope of this regioselective palladium-catalysed alcohol oxidation towards oligoglucosides. The aim of this study was to investigate the compatibility of the oxidation with the presence of an azide. This makes it a powerful tool to prepare orthogonal bis-functionalized oligosaccharides, bearing both an azide and ketone functionality. Like PEG chains, (1→4) linked glucans, have shown to stabilize proteins,[9] and may serve as spacers for the preparation of protein-drug conjugates. In addition, 1→4 linked glucans, may well be used as molecular rulers and building blocks for copolymers.

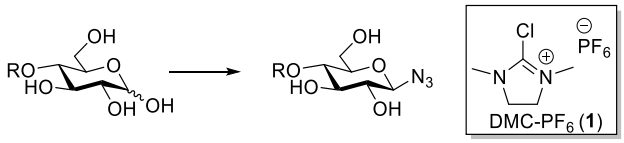
2.2 Results and discussion

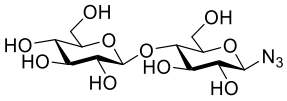
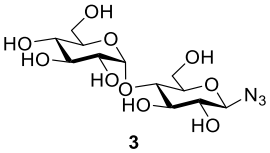
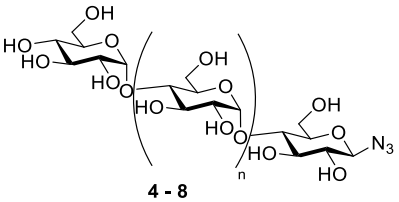
2.2.1. Preparation of the substrates

The synthesis of the required glycosyl azides as starting materials for the catalytic oxidation was carried out according to literature. Shoda recently pioneered the application of 2-chloro-1,3-dimethylimidazolinium chloride

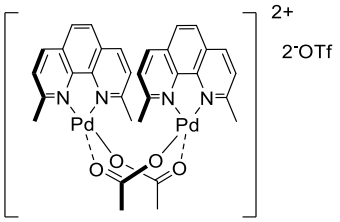
(DMC) for the synthesis of anomeric azides in unprotected oligosaccharides.[10–14] For scalability reasons, we aimed to replace the described preparative HPLC purification. Silica gel chromatography with 10 – 15% water in acetonitrile as the eluent provided NMR-pure glucosyl azides, but all subsequent oxidation experiments failed to give conversion. Further study showed that 1,3-dimethyl-imidazolidin-2-one did not hamper the oxidation reaction but small amounts of NaCl, NaBr, KI and NaN_3 led to complete inhibition. Apparently, salts in the reaction mixture co-eluted during purification.[15] To lower the amount of chloride present, DMC chloride was replaced by DMC-PF_6 **1**. Additional advantage is that this reagent is less hygroscopic and therefore easier to handle. As an alternative, it is also possible to directly use ADMC, in which the azide is already introduced in the activating agent.[16] The key measure, however, turned out to be the application of column chromatography on charcoal. Improving upon the procedure of Whistler et al,[17] a ratio of 10 : 1 (w/w charcoal to product) and elution with water followed by a gradient of ethanol/water turned out sufficient to purify the glucosyl azides on preparative scale. With this purification method in hands, we prepared the

Table 1. Preparation of oligoglycosyl azides.



Entry	Product	Yield (%)
1[a]	 **2**	81
2[a]	 **3**	74
3-7[b]	 **4 - 8**	**4:** n=1 : 81 **5:** n=2: 76 **6:** n=3 : 74 **7:** n=4 : 69 **8:** n=5 : 76

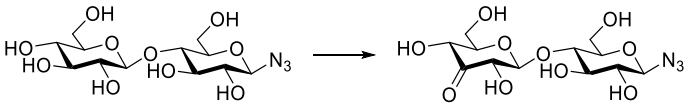
Reaction conditions: a) 3 equiv of DMC-PF_6 **1**, 9 equiv of NaN_3, 10 equiv of $^iPr_2NEt$, 0.25M H_2O, r.t. 1.5h b) 7 equiv of DMC-PF_6 **1**, 50 equiv of NaN_3, 15 equiv of $^iPr_2NEt$, 0.1M H_2O., r.t. 3h



[(neocuproine)PdOAc]$_2$OTf$_2$ (**9**)

Figure 1. Cationic palladium complex.

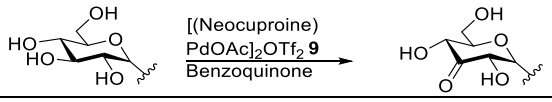
desired range of maltosyl to maltoheptaosyl azides (**2** – **8**, see Table 1). We subsequently subjected the obtained glucosyl azides to palladium catalysed regioselective oxidation. Hitherto, its compatibility with azides had not been studied, and the substrate scope was limited to mono- and disaccharides. β-D-Cellobiosyl azide **2** was selected as starting point for the oxidation due to its straightforward comparison to the reported oxidation of methyl *α*-D-cellobioside. When **2** was reacted with 0.5 mol% of catalyst **9** (Figure 1), no conversion was observed. An increased catalyst loading to 7.5 mol%, however, led to full conversion (Scheme 1). Ketone **10** was readily purified by charcoal column chromatography to give 61% isolated yield (Table 2, entry 1). According to NMR, the C3-OH of the non-azido glucose residue had been oxidized selectively. The H2 and H4 of this ring shifted significantly downfield, separating them from the other signals in the ^{1}H-NMR and showed a long-range coupling (~1.6 Hz). Furthermore, the H2 of the oxidized ring coupled to the H1 of the *O*-glycoside and not to the H1 of the *N*-glycoside. β-D-3-Ketomaltosyl azide **11** could also be produced in this fashion in a similar yield (Table 2, entry 2). Apparently, the Pd-catalysed oxidation is compatible with anomeric azides although an increased amount of catalyst is required. In the same way, β-D-maltotriosyl azide was oxidized, which provided **12** in 60% isolated yield. Although ^{1}H-NMR readily identified oxidation at a C3 position, identifying which glucose residue had been oxidized proved to be more challenging. An empirical study by Bock and coworkers[18,19] had revealed that the C4 carbon involved in a glycosidic bond has a distinct downfield shift in ^{13}C-NMR (~77-80 ppm) compared to the other carbon signals (55-76 ppm, apart from the anomeric

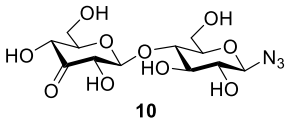
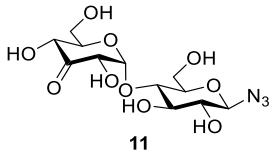
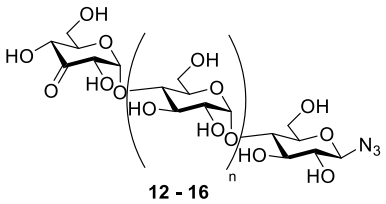


Scheme 1. Oxidation of β-D-cellobiosyl azide **2.** Reaction conditions: 7.5 mol% of [(neocuproine)PdOAc]$_2$OTf$_2$, 3 equiv benzoquinone, 0.3 M in DMSO/dioxane 1/4, r.t. 7 h.

carbons). The straightforward assignment of the H4 next to the ketone in ^{1}H-NMR enabled identification of the corresponding carbon signal with HMQC, see Figure 3. The signal of this C4 appeared at 71.8 ppm, identifying the carbon as a CHOH moiety, which confirmed that oxidation had taken place at the terminal non-azido end. To verify this analysis, the C4 of the glucosyl azide moiety was also determined. Using TOCSY and COSY NMR techniques H4 of this ring was readily identified. As described above, the corresponding C4 could be found using HMQC, giving a signal typically around 77-80 ppm (see Figure 2). Furthermore, the chemical shifts of the synthesized β-D-3-ketomaltotriosyl azide were in agreement with the reported values of the oxidation at the terminal C3 position of maltotriose.[20] With selective oxidation on the terminal glucose residue in this trisaccharide established, the scope was extended to even larger oligosaccharides. β-D-maltotetraosyl azide was oxidized to **13** in 38% isolated yield upon increasing the catalyst loading to 15 mol%. With this protocol, we demonstrated that azido-β-1,4-glucans ("azido oligomaltoses") up to maltoheptaose were readily and in high selectivity converted into their C3-keto

Table 2. Regioselective oxidation of oligomaltosyl azides.



Entry	Product	Yield (%)
1	 **10**	61
2	 **11**	59
3-7[a]	 **12 - 16**	**12** n=1: 60 **13** n=2: 38 **14** n=3: 30 **15** n=4: 30 **16** n=5: 47

Reaction conditions: 7.5 mol% of [(neocuproine)PdOAc]$_2$OTf$_2$ **9**, 3 equiv of benzoquinone, 0.3 M in DMSO/dioxane 1:4, r.t. 7 h. a) 15 mol% of [(neocuproine)PdOAc]$_2$OTf$_2$ **9**, 3 equiv of benzoquinone, 0.15 M in DMSO.

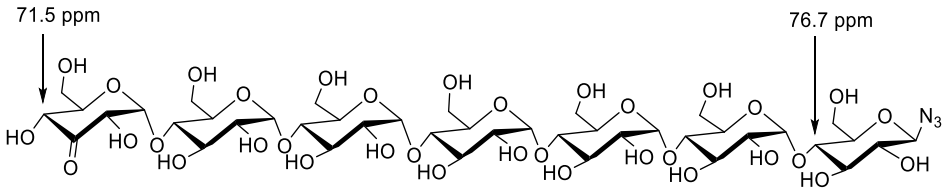


Figure 2. β-D-3-ketomaltoheptaosyl azide + ^{13}C-NMR shifts.

derivatives (see Table 2, entry 3-7 and Figure 2). NMR analysis shows that in all cases oxidation takes place selectively at the C3-position of the terminal non-reducing glucose unit. The reaction proceeds with exceptional regioselectivity and only very small amounts of regio-isomers and products resulting from overoxidation were observed in the crude reaction mixture. To indicate the level of selectivity, in Figure 4 there is an overlay of the crude and isolated ^{1}H-NMR spectrum of β-D-3-ketomaltoheptaosyl azide. Although the reactions proceed with full conversion of the starting material, purification of these highly polar compounds is challenging. Charcoal column chromatography effectively removed the impurities, however resulting in a somewhat decreased isolated yield. Upon prolonged reaction, overoxidation on different positions is observed.

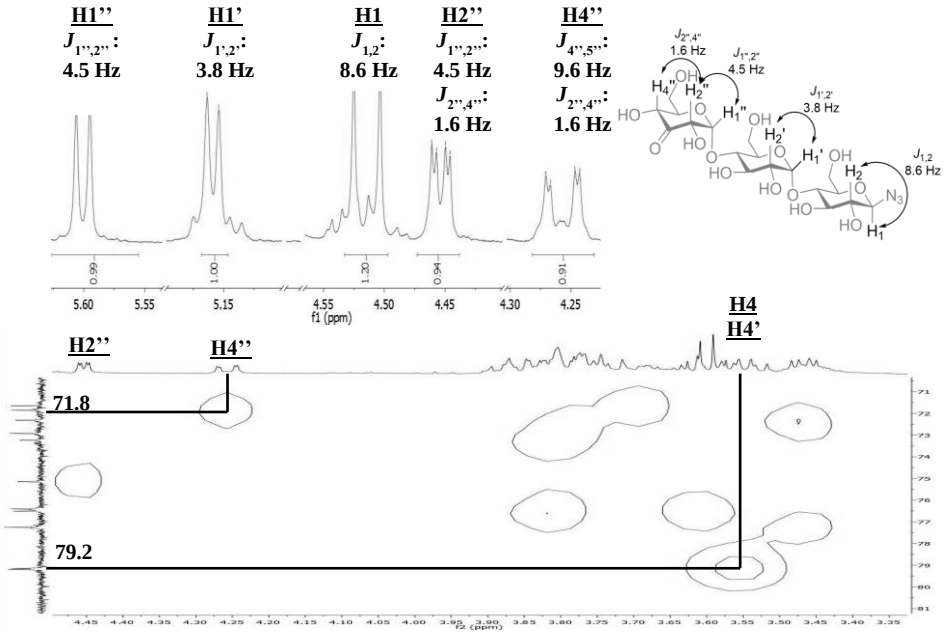


Figure 3. NMR analysis of β-D-3-ketomaltotriosyl azide **12** Relevant sections of the spectrum are shown. HMQC: Correlation of H4'' with the corresponding carbon signal. H4 and H4' were determined via COSY/TOCSY.

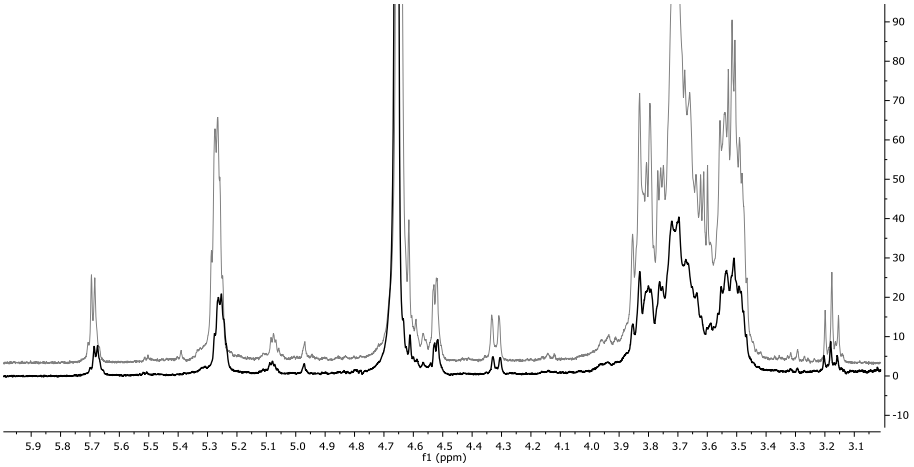
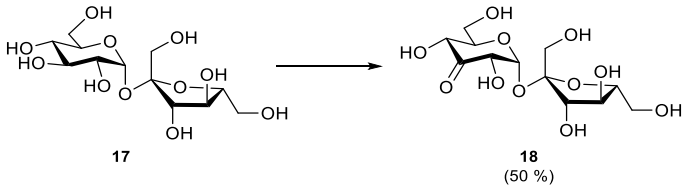


Figure 4. ^{1}H-NMR of β-D-3-ketomaltoheptaosyl azide **16**. Top spectrum is of the crude reaction mixture. Bottom spectrum is after charcoal purification.

As an indication of the extreme selectivity of the reaction, the 47% yield in the oxidation of β-D-maltoheptaosyl azide translates in a selectivity ratio of >10. Why only the terminal C3-OH is oxidized will be further discussed in Chapter 4.

To further expand the scope of the oxidation reaction, readily available sucrose, although not a 1,4-linked glucan, was studied as it consists of glucose 1,1-linked to fructose. The reaction was monitored by quantitative ^{1}H-NMR (Q-NMR). The reaction gave 50% of the expected 3-keto sucrose (**18**) together with several side products in smaller amounts (Scheme 2).



Scheme 2. Oxidation of sucrose. Reaction conditions: 2.5 mol% of [(neocuproine)PdOAc]$_2$OTf$_2$, 3 equiv benzoquinone, 0.3 M in DMSO-d6, r.t. 1 h. Conversion determined by Q-NMR.

2.2.2 Bis-functionalization of a mutant of 4-oxalocrotonate tautomerase

Bis-functionalized oligosaccharides, in particular oligo-maltoses, are potentially highly effective molecular rulers and spacers.[21–23] Oligomaltoses share a high water solubility with poly-ethylene glycol (PEG), but contrary to the latter they have a well-defined length and stiffness due to their internal structure.[24] As the two introduced functional groups, a ketone and an azide, are orthogonal to

each other, and bio-orthogonal. One of these handles can be used for the glycosylation of a protein, and the second one for subsequent modification of the glycoprotein conjugate with a molecule of interest (Figure 5). To validate the feasibility of this application, such a protein-glycan conjugate was prepared. A cysteine mutant of 4-oxalocrotonate tautomerase, denoted 4-OT R61C-1, coupled to a terminal alkyne at the cysteine residue via a maleimide linker, was selected as a model protein.[25] We decided to ligate biotin hydrazide to the ketone functionality of the saccharide residue, for straightforward visualization of the bisfunctionalized construct by Western blotting. After hydrazone formation, the

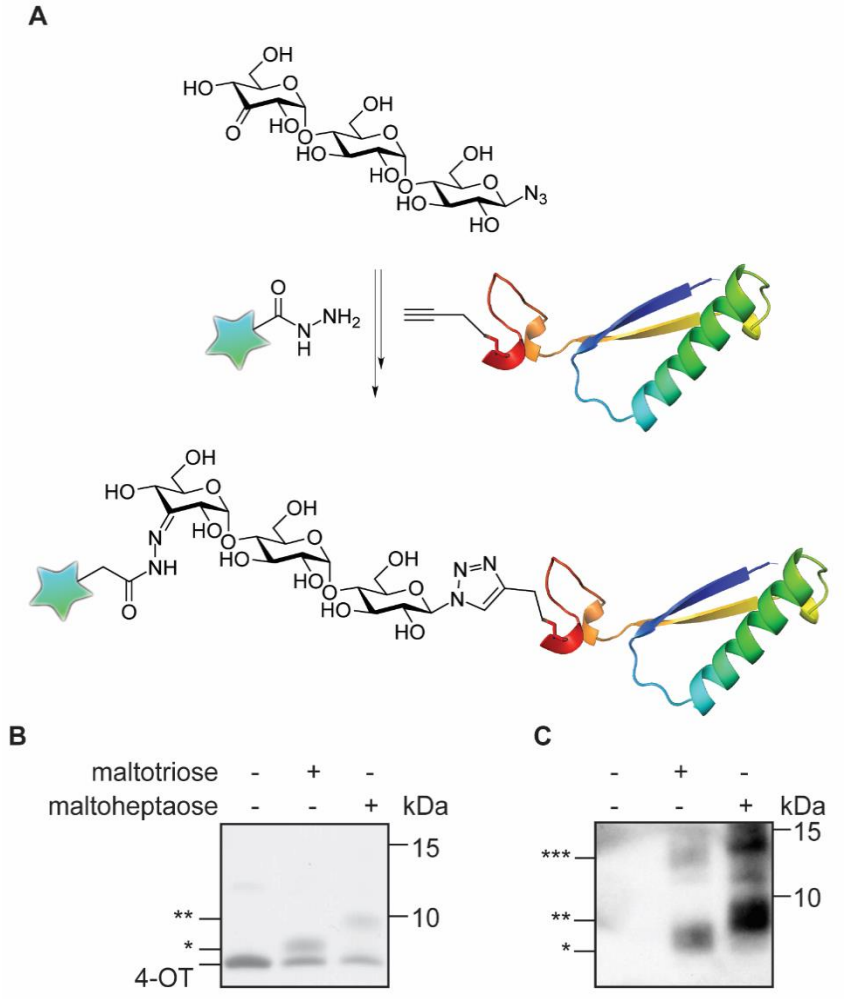


Figure 5. A: Introduction of a protein-alkyne and a biotin hydrazide onto β-D-ketomaltoheptaosyl azide. B: Tricine SDS Page, visualized by Coomassie Brilliant Blue stain. C: Western Blot using Strep-HRP and ECL (* 4-OT modified with maltotriose biotin, ** 4-OT modified with maltoheptaose biotin, *** 4-OT oligomers).

modified oligosaccharides β-D-maltotriosyl azide-biotin and β-D-maltoheptaosyl azide-biotin were incubated with the protein in the presence of $CuSO_4$/tris (3-hydroxypropyltriazolylmethyl)amine and sodium ascorbate (Figure 5A). As a control, the same reaction was performed in the absence of the saccharides. Tricine SDS-PAGE analysis of the conjugation reaction visualized by Coomassie stain showed in the cases with saccharide present the appearance of new bands. The molecular weight of these bands is increased compared to the unmodified protein and corresponds with the respective functionalization of 4-OT with β-D-maltotriosyl azide-biotin and β-D-maltoheptaosyl azide-biotin (Figure 5B). In the control reaction, this particular shift was not observed, further confirming that these new bands originate from the biotin-carbohydrate-protein adduct. To verify the bis-functionalization of the oligosaccharide, we visualized the biotinylated protein adducts via Western blotting. To this end, the biotin-carbohydrate-protein adduct was treated with streptavidin protein that is covalently conjugated to horseradish peroxidase (HRP) enzyme. The terminal biotin moiety binds to the streptavidin and the conjugated HRP provides enzyme activity for detection by reduction of luminol. As depicted in Figure 5C, a strong chemi-luminescence signal arising from the protein-oligosaccharide-biotin conjugate was observed at the expected molecular weight. Inversion of the ligation steps, e.g. first ligation of the protein to the azido carbohydrate, followed by biotinylation was also effective, albeit with a lower efficiency.

2.3 Conclusions

In conclusion, palladium-catalysed alcohol oxidation allows the regioselective modification of azido-β-(1→4)-glucans, in casu azido oligomaltoses. The chemo- and regioselectivity of the catalyst system $[(neocuproine)PdOAc]_2OTf_2$ is extremely high and unprecedented; in azido maltoheptaose one secondary hydroxyl group is oxidized in the presence of 7 primary and 15 nearly identical secondary hydroxyl groups to provide the product in 47% isolated yield. Oxidation takes place at the C3-position of the terminal residue at the non-azido end. As the oxidation is compatible with an azide functionality in the substrate this allows the synthesis of well-defined orthogonal bisfunctionalized (1→4)-linked glucans. Column chromatography on charcoal allows purification on a synthetically useful scale. Among the many foreseeable applications as biocompatible spacers, molecular rulers, and building blocks for copolymers, it has been demonstrated that a protein-oligomaltose adduct can be prepared and subsequently visualized via biotinylation at the other terminus of the glycan.

2.4 Experimental section

2.4.1 General information

Solvents and Reagents

All solvents used for reaction, extraction, filtration and chromatography were of commercial grade, and used without further purification. Maltotetraose was purchased from Carbosynth, maltopentaose up to maltoheptaose was purchased from TCI Europe. DMC-Cl PF_6 was purchased from AK Scientific. Benzoquinone was sublimed prior to using. $[(Neocuproine)PdOAc]_2OTf_2$ was prepared according to literature procedure.[26]

Analysis

TLC was performed on Merck silica gel 60, 0.25 mm plates and visualization was done by staining with anisaldehyde reagent (a mixture of acetic acid (300 ml), H_2SO_4 (6 ml), anisaldehyde (3 ml)) or potassium permanganate stain (a mixture of $KMnO_4$ (3 g), K_2CO_3 (10 g), water (300 mL)).

^1H-, ^{13}C-, APT-, COSY-, TOCSY and HMQC-NMR were recorded on a Varian AMX400 spectrometer (400, 100 MHz, respectively) using CD_3OD or D_2O as solvent. Chemical shift values are reported in ppm with the solvent resonance as the internal standard (CD_3OD: δ 3.31 for ^1H, δ 49.15 for ^{13}C; D_2O: δ 4.80 for ^1H). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, t = triplet, appt = apparent triplet, q =quartet, m = multiplet), coupling constants *J* (Hz), and integration. High Resolution Mass Spectroscopy measurements were performed using a ThermoScientific LTQ OribitrapXL spectrometer. Optical rotations were measured on a Schmidt + Haensch polarimeter (Polartronic MH8) with a 10 cm cell (c given in g/100 mL). Infrared (IR) data were recorded on a Perkin Elmer UATR Spectrum Swo FT-IR spectrometer. Absorbance frequencies are reported in reciprocal centimeters (cm^{-1})

2.4.2 Purification: General method for purification on charcoal.

Preparation of a charcoal column:

Solid loading cartridges (solid loading cartridges for a Grace Reveleris automatic column system) were used to perform the column chromatography. One frit was placed on the bottom of the cartridge, followed by the desired amount of charcoal (DARCO® G60 activated charcoal, 100 mesh particle size, equal to 10 times the amount of starting material used) Then a second frit was added and pressed firmly together. The charcoal column was washed with ~5 column volumes (CV) of water and stored wet until use (Figure 6A).

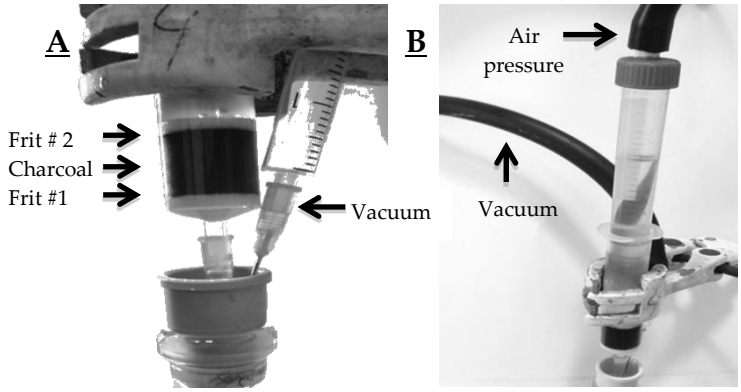


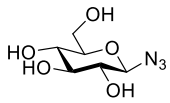
Figure 6. A: close up charcoal column (~1 g) B: charcoal column vacuum + air pressure, adapter made from a 15 ml Greiner tube, bottom removed + hole in cap for connection to the air pressure.

Performing charcoal column chromatography (See Figure 6A and 6B):
Charcoal column chromatography is performed with negative pressure (vacuum) in combination with air pressure to maintain an acceptable flow rate (depending on the size of the column, only vacuum can be sufficient). Before loading, traces of organic solvents are removed *in vacuo* from the sample. The remaining aqueous solution is directly loaded onto the prepared (and washed) charcoal column, and elution starts with pure water to elute salts (if applicable, the presence of salts in eluted fractions is indicated with 0.1 M $AgNO_{3(aq)}$). Upon complete elution of the salts, a gradient of ethanol/water is used to elute the desired product (2 – 4 CV per 2% increase, followed by TLC monitoring for elution of product(s)). When the desired product starts to elute, the polarity of the eluent can be further decreased (to 20 - 30% ethanol/water) to speed up the elution. The fractions containing the product are evaporated or freeze-dried to yield the pure product.
For larger molecules, such as maltotetraose and higher oligos, tert-butanol is more efficient than ethanol.

For purification of the oxidized products, the charcoal chromatography is performed with a small frit of celite on top of the column, to retain the precipitated hydroquinone. Upon complete loading of the product, this frit is removed from the column to ensure an acceptable flow rate, and the elution is continued as stated above.

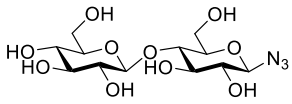
2.4.3. Synthesis of glucosyl azides

β-D-glucosyl azide (19)



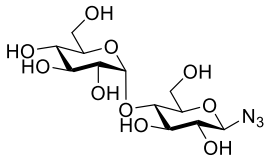
To a solution of glucose (500 mg, 2.78 mmol, 1 eq), sodium azide (1.8 g, 27.8 mmol, 10 eq) and *N*,*N*-diisopropylethylamine (4.4 ml, 25 mmol, 9 eq) in water (4.4 ml) at 0 °C was added DMC-PF_6 (2.3 g, 8.33 mmol, 3 eq). The resulting mixture was stirred at 0 °C until complete conversion of starting material was observed on TLC (eluent: 15% H_2O/CH_3CN). Upon complete conversion, the solution was washed with dichloromethane (3 x 50 ml). The resulting water layer was purified by charcoal column chromatography (7% EtOH/H_2O eluted the desired product) and concentrated *in vacuo* to yield 460 mg (2.24 mmol, 81%) of pure β-D-glucosyl azide. ^{1}H-NMR (400 MHz, CD_3OD) δ 4.50 (d, *J* = 8.6 Hz, 1H), 3.87 (dd, *J* = 12.1, 2.1 Hz, 1H), 3.68 (dd, *J* = 12.1, 5.3 Hz, 1H), 3.42 – 3.27 (m, 3H), 3.14 (appt, *J* = 8.9 Hz, 1H). ^{13}C-NMR (101 MHz, CD_3OD) δ 91.9, 80.0, 78.0, 74.7, 71.0, 62.5.
Characterization matches literature.[14]

β-D-cellobiosyl azide (2)



To a solution of cellobiose (100 mg, 0.292 mmol, 1 eq), sodium azide (190 mg, 2.92 mmol, 10 eq) and *N*,*N*-diisopropylethylamine (460 µl, 2.63 mmol, 9 eq) in water (1.1 ml) at room temperature was added DMC-PF_6 (245 mg, 0.88 mmol, 3 eq). The resulting mixture was stirred at room temperature until TLC indicated full conversion of starting material (eluent: 15% H_2O/CH_3CN). Upon complete conversion, the solution was washed with dichloromethane (3 x 20 ml). The resulting water layer was purified by charcoal column chromatography (14% EtOH/H_2O eluted the desired product) and concentrated *in vacuo* to yield 87 mg (0.237 mmol, 81%) of pure β-D-cellobiosyl azide. ^{1}H-NMR (400 MHz, CD_3OD) δ 4.55 (d, *J* = 8.7 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 3.95 – 3.83 (m, 3H), 3.66 (dd, *J* = 11.9, 5.5 Hz, 1H), 3.62 – 3.48 (m, 3H), 3.41 – 3.29 (m, 3H, overlapping with CD_3OD), 3.26 – 3.18 (m, 2H). ^{13}C-NMR (101 MHz, CD_3OD) δ 104.5, 91.8, 80.0, 78.5, 78.0, 77.8, 76.4, 74.8, 74.4, 71.3, 62.4, 61.6.
Characterization matches literature.[14]

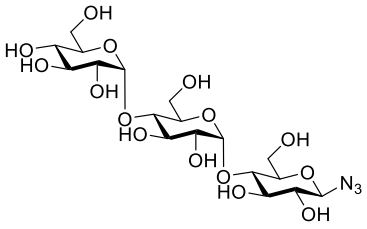
β-D-maltosyl azide (3)



To a solution of maltose monohydrate (100 mg, 0.278 mmol, 1 eq), sodium azide (180 mg, 2.78 mmol, 10 eq) and *N*,*N*-diisopropylethylamine (440 µl, 2.50 mmol, 9 eq) in water (1.1 ml) at room temperature was added DMC-PF_6 (230 mg, 0.83 mmol, 3 eq). The resulting mixture was stirred at room temperature until TLC indicated full conversion of

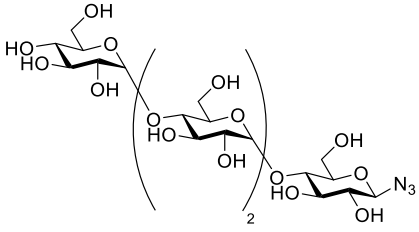
starting material (eluent: 20% H_2O/CH_3CN). Upon complete conversion, the solution was washed with dichloromethane (3 x 20 ml). The resulting water layer was purified by charcoal column chromatography (11% $EtOH/H_2O$ eluted the desired product) and concentrated *in vacuo* to yield 76 mg (0.207 mmol, 74%) of pure β-D-maltosyl azide. ^{1}H-NMR (400 MHz, CD_3OD) δ 5.18 (d, *J* = 3.8 Hz, 1H), 4.54 (d, *J* = 8.6 Hz, 1H), 3.9 (dd, *J* = 12.39, 1.98 Hz, 1H), 3.86 – 3.80 (m, 2H), 3.71 – 3.63 (m, 3H), 3.63 – 3.54 (m, 2H), 3.50 (td, *J* = 5.2, 4.8, 1.9 Hz, 1H), 3.46 (dd, *J* = 9.7, 3.7 Hz, 1H), 3.32 – 3.24 (m, 1H, overlapping with CD_3OD), 3.20 (appt, *J* = 8.9 Hz, 1H). ^{13}C NMR (101 MHz,CD_3OD) δ 102.8, 91.9, 80.5, 78.7, 77.8, 75.0, 74.7, 74.3, 74.1, 71.4, 62.7, 61.9.
Characterization matches literature.[14]

β-D-maltotriosyl azide (4)



To a solution of maltotriose (93% purity, 100 mg, 0.184 mmol, 1 eq), sodium azide (120 mg, 1.84 mmol, 10 eq) and *N*,*N*-diisopropylethylamine (290 µl, 1.66 mmol, 9 eq) in water (0.75 ml) at room temperature was added DMC-PF_6 (155 mg, 0.55 mmol, 3 eq). The resulting mixture was stirred at room temperature until TLC indicated full conversion of starting material (eluent: 30% H_2O/CH_3CN). Upon complete conversion, the solution was washed with dichloromethane (3 x 20 ml). The resulting water layer was purified by charcoal column chromatography (12% $EtOH/H_2O$ eluted the desired product) and concentrated *in vacuo* to yield 79 mg (0.149 mmol, 81%) of pure β-D-maltotriosyl azide. ^{1}H-NMR (400 MHz, CD_3OD) δ 5.19 (d, *J* = 3.8 Hz, 1H), 5.16 (d, *J* = 3.8 Hz, 1H), 4.55 (d, *J* = 8.6 Hz, 1H), 3.94 – 3.73 (m, 7H), 3.72 – 3.42 (m, 9H), 3.35 – 3.24 (m, 1H, overlapping with CD_3OD), 3.21 (appt, *J* = 8.9 Hz, 1H) ^{13}C NMR (101 MHz,CD_3OD) 102.8, 102.6, 91.9, 81.2, 80.5, 78.6, 77.8, 75.0, 74.9, 74.7, 74.3, 74.1, 73.7, 73.3, 71.4, 62.6, 62.1, 62.0.
Characterization matches literature..[14]

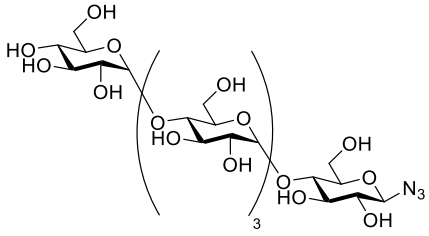
β-D-maltotetraosyl azide (5)



To a solution of maltotetraose (100 mg, 0.150 mmol, 1 eq), sodium azide (490 mg, 7.5 mmol, 50 eq) and *N*,*N*-diisopropylethylamine (390 µl, 2.25 mmol, 15 eq) in water (1.5 ml) at room temperature was added DMC-PF_6 (210 mg, 0.75 mmol, 5 eq). The resulting mixture was stirred at room temperature until TLC indicated full conversion of starting

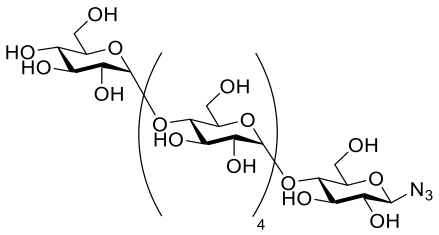
material (eluent: 30% H_2O/CH_3CN). Upon complete conversion, the solution was washed with dichloromethane (3 x 20 ml). The resulting water layer was purified by charcoal column chromatography (3 % $^tBuOH/H_2O$ eluted the desired product) and concentrated *in vacuo* to yield 79 mg (0.114 mmol, 76%) of pure β-D-maltotetraosyl azide. ^1H NMR (400 MHz, D_2O) δ 5.45 – 5.38 (m, 3H), 4.79 (d, *J* = 8.7 Hz, 1H), 4.01 – 3.91 (m, 3H), 3.92 – 3.77 (m, 9H), 3.77 – 3.55 (m, 10H), 3.43 (appt, *J* = 9.4 Hz, 1H), 3.32 (appt, *J* = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 99.7, 99.6, 99.3, 89.8, 76.8, 76.6, 76.4, 76.2, 76.1, 73.3, 73.2, 72.8, 72.6, 71.7, 71.5, 71.4, 71.1, 69.2, 60.4, 60.3. (4 signals are missing due to severe overlap).
Characterization matches literature.[14]

β-D-maltopentaosyl azide (6)



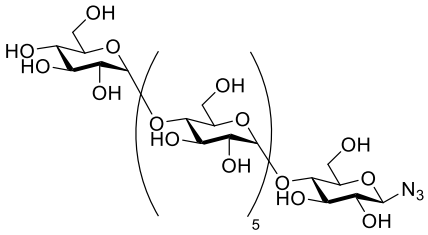
To a solution of maltopentaose (109 mg, 0.132 mmol, 1 eq), sodium azide (428 mg, 6.58 mmol, 50 eq) and *N*,*N*-diisopropylethylamine (350 µl, 1.97 mmol, 15 eq) in water (1.3 ml) at room temperature was added DMC-PF_6 (183 mg, 0.658 mmol, 5 eq). The resulting mixture was stirred at room temperature until TLC indicated full conversion of starting material (eluent: 30% H_2O/CH_3CN). Upon complete conversion, the solution was washed with dichloromethane (3 x 20 ml). The resulting water layer was purified by charcoal column chromatography (3.5 % $^tBuOH/H_2O$ eluted the desired product) and concentrated *in vacuo* to yield 83 mg (0.097 mmol, 74%) of pure β-D-maltopentaosyl azide. ^1H NMR (400 MHz, D_2O) δ 5.28 – 5.24 (m, 4H), 4.62 (d, *J* = 8.9 Hz, 1H), 3.86 – 3.76 (m, 4H), 3.75 – 3.63 (m, 12H), 3.60 – 3.42 (m, 12H), 3.28 (appt, *J* = 9.4 Hz, 1H), 3.17 (appt, *J* = 9.1 Hz, 1H).^{13}C NMR (101 MHz, D_2O) δ 99.7, 99.5, 99.5, 99.4, 89.8, 76.8, 76.7, 76.6, 76.4, 76.2, 76.1, 73.3, 73.2, 73.2, 72.8, 72.6, 72.6, 71.6, 71.5, 71.4, 71.1, 69.2, 60.4, 60.4, 60.3. (5 signals are missing due to severe overlap).
Characterization matches literature[14]

β-D-maltohexaosyl azide (7)



To a solution of maltohexaose (124 mg, 0.125 mmol, 1 eq), sodium azide (407 mg, 6.26 mmol, 50 eq) and *N*,*N*-diisopropylethylamine (325 µl, 1.875 mmol, 15 eq) in water (1.25 ml) at room temperature was added DMC-PF_6 (174 mg, 0.625 mmol, 5 eq). The resulting mixture was stirred at room temperature until TLC indicated full conversion of starting material (eluent: 30% H_2O/CH_3CN). Upon complete conversion, the solution was washed with dichloromethane (3 x 15 ml). The resulting water layer was purified by charcoal column chromatography (3.75 % $^tBuOH/H_2O$ eluted the desired product) and concentrated *in vacuo* to yield 87 mg (0.086 mmol, 69%) of pure β-D-maltohexaosyl azide. ^1H NMR (400 MHz, D_2O) δ 5.27 (m, 5H), 4.62 (d, *J* = 9.1 Hz, 1H), 3.87 – 3.77 (m, 5H), 3.77 – 3.62 (m, 15H), 3.62 – 3.42 (m, 14H), 3.28 (appt, *J* = 9.3 Hz, 1H), 3.17 (appt, *J* = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 99.7, 99.5, 99.5, 99.5, 99.4, 89.8, 76.8, 76.7, 76.7, 76.6, 76.6, 76.4, 76.2, 76.1, 73.3, 73.2, 73.2, 72.8, 72.6, 71.6, 71.5, 71.4, 71.1, 71.1, 69.2, 60.4, 60.3. (9 signals are missing due to severe overlap).
Characterization matches literature.[14]

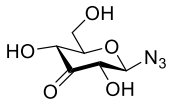
β-D-maltoheptaosyl azide (8)



To a solution of maltoheptaose (80% purity, 100 mg, 0.0694 mmol, 1 eq), sodium azide (225 mg, 3.5 mmol, 50 eq) and *N*,*N*-diisopropylethylamine (180 µl, 1.04 mmol, 15 eq) in water (0.7 ml) at room temperature was added DMC-PF_6 (100 mg, 0.347 mmol, 5 eq). The resulting mixture was stirred at room temperature until TLC indicated full conversion of starting material (eluent: 35% H_2O/CH_3CN). Upon complete conversion, the solution was washed with dichloromethane (3 x 20 ml). The resulting water layer was purified by charcoal column chromatography (3.5 % $^tBuOH/H_2O$ eluted the desired product) and concentrated *in vacuo* to yield 62 mg (0.0526 mmol, 76%) of pure β-D-maltoheptaosyl azide. ^1H NMR (400 MHz, D_2O) δ 5.43 – 5.38 (m, 6H), 4.76 (d, *J* = 9.6 Hz, 1H), 4.01 – 3.92 (m, 6H), 3.90 – 3.77 (m, 17H), 3.77 – 3.55 (m, 17H), 3.43 (appt, *J* = 9.4 Hz, 1H), 3.31 (appt, *J* = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 99.7, 99.6, 99.4, 89.9, 76.7, 76.6, 76.4, 76.2, 76.1, 73.3, 73.2, 73.2, 72.8, 72.6, 71.7, 71.5, 71.4, 71.1, 71.1, 69.2, 60.4, 60.4, 60.4, 60.3. (18 signals are missing due to severe overlap). Characterization matches literature.[14]

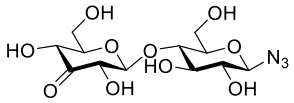
2.4.4 Synthesis of keto-glycosyl azides

β-D-3-ketoglucosyl azide (20)



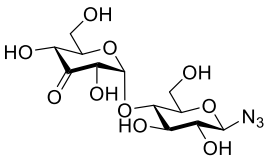
β-D-glucosyl azide **19** (50 mg, 0.243 mmol, 1 eq) was dissolved in a dioxane/DMSO mixture (4:1, 800 µl, 0.3 M), before benzoquinone (80 mg, 0.731 mmol, 3 eq) and [(2,9-dimethyl-1,10-phenanthroline)-Pd(µ-OAc)]$_2$(OTf)$_2$ (20 mg, 18 µmol, 7.5 mol%) were added. The reaction was stirred at room temperature till complete consumption of starting material (indicated by TLC (eluent: 15% MeOH/CH_2Cl_2)). Upon completion, the reaction mixture was diluted with 10% EtOH/H_2O (7 ml) and the resulting aqueous solution was flushed over a charcoal column and concentrated *in vacuo* to yield β-D-3-ketoglucosyl azide. *(**18** is highly unstable, flushing over charcoal yielded the product with minor amounts of degradation. Due to this instability a yield of this reaction could not be determined, **18** was only characterized by ^{1}H-NMR.)* ^{1}H NMR (400 MHz, CD_3OD) δ 4.66 (d, *J* = 8.7 Hz, 1H), 4.29 (dd, *J* = 10.2, 1.7 Hz, 1H), 4.13 (dd, *J* = 8.7, 1.6 Hz, 1H), 3.95 (dd, *J* = 12.2, 2.1 Hz, 1H), 3.81 (dd, *J* = 12.4, 4.9 Hz, 1H), 3.48 (ddd, *J* = 10.3, 4.7, 2.0 Hz, 1H).

β-D-3-ketocellobiosyl azide (10)



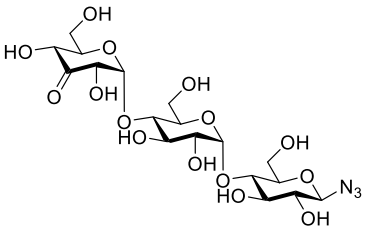
β-D-cellobiosyl azide **2** (40 mg, 0.109 mmol, 1 eq) was dissolved in a dioxane/DMSO mixture (4:1, 370 µl, 0.3 M), before benzoquinone (35 mg, 0.327 mmol, 3 eq) and [(2,9-dimethyl-1,10-phenanthroline)-Pd(µ-OAc)]$_2$(OTf)$_2$ (9 mg, 8.6 µmol, 7.5 mol%, added in 3 portions over 6 h) were added. The reaction was stirred at room temperature till complete consumption of starting material (indicated by TLC (eluent: $CHCl_3$:MeOH:EtOAc:H_2O 2:2:4:0.75)). Upon completion, the reaction mixture was diluted with H_2O (7 ml) and the resulting aqueous solution was purified by charcoal column chromatography (12% EtOH/H_2O eluted the desired product). The product was freeze-dried to yield 26 mg (0.071 mmol, 65%) of an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 4.56 (d, *J* = 8.7 Hz, 1H), 4.56 (d, *J* = 7.9 Hz, 1H), 4.24 (dd, *J* = 10.2, 1.7 Hz, 1H), 4.18 (dd, *J* = 8.0, 1.8 Hz, 1H), 3.97 – 3.86 (m, 3H), 3.78 (dd, *J* = 12.1, 5.0 Hz, 1H), 3.72 – 3.65 (m, 1H), 3.58 (t, *J* = 9.0 Hz, 1H), 3.52 (ddd, *J* = 9.7, 3.7, 2.3 Hz, 1H), 3.38 (ddd, *J* = 10.1, 5.0, 2.1 Hz, 1H), 3.21 (appt, *J* = 8.9 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 206.6, 105.7, 91.9, 79.6, 78.6, 78.2, 78.2, 76.4, 74.5, 73.4, 62.3, 61.3. HRMS (ESI) calculated for $C_{12}H_{19}O_{10}N_3Na$ ($[M+Na]^+$): 388.096, found: 388.096 IR νmax/cm^{-1}: 3368 (OH), 2888 (C-H), 2118 (N_3), 1734 (C=O), 1028 (C-O) $[\alpha]_D^{20}$ = -20 (c 0.6, H_2O)

β-D-3-ketomaltosyl azide (11)



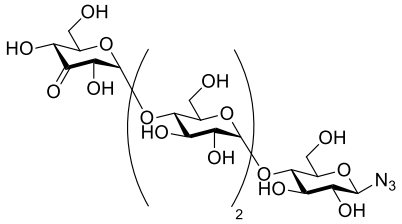
β-D-maltosyl azide **3** (76 mg, 0.207 mmol, 1 eq) was dissolved in a dioxane/DMSO mixture (4:1, 700 µl, 0.3 M), before benzoquinone (67 mg, 0.620 mmol, 3 eq) and [(2,9-dimethyl-1,10-phenanthroline)-Pd(µ-OAc)]$_2$(OTf)$_2$ (16 mg, 15.5 µmol, 7.5 mol%, added in 3 portions over 6h) were added. The reaction was stirred at room temperature till complete consumption of starting material (indicated by TLC (eluent: $CHCl_3$:MeOH:EtOAc:H_2O 2:2:4:0.75)). Upon completion, the reaction mixture was diluted with H_2O (14 ml) and the resulting aqueous solution was purified by charcoal column chromatography (7% EtOH/H_2O eluted the desired product). The product was freeze dried to yield 46 mg (0.122 mmol, 59%) as an off-white solid. (contains ~10% hydroquinone by NMR integration, isolated yield corrected for this value). ^{1}H NMR (400 MHz, CD_3OD) δ 5.64 (d, *J* = 4.5 Hz, 1H), 4.50 (d, *J* = 8.7 Hz, 1H), 4.46 (dd, *J* = 4.5, 1.5 Hz, 1H), 4.26 (dd, *J* = 9.5, 1.6 Hz, 1H), 3.91 – 3.76 (m, 5H), 3.64 – 3.58 (m, 2H), 3.43 (ddd, *J* = 9.2, 4.4, 1.9 Hz, 1H), 3.17 (appt, *J* = 8.7 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 207.0, 104.7, 91.9, 79.7, 78.4, 77.9, 77.6, 76.5, 74.4, 73.3, 62.5, 61.8. HRMS (ESI) calculated for $C_{12}H_{19}O_{10}N_3Na$ ([M+Na]$^+$): 388.096, found: 388.096 IR νmax/cm^{-1}: 3343 (OH), 2928 (C-H), 2118 (N_3), 1736 (C=O), 1028 (C-O) $[\alpha]_D^{20}$ = + 89.6 (c 1.00, H_2O)

β-D-3-ketomaltotrioside (12)



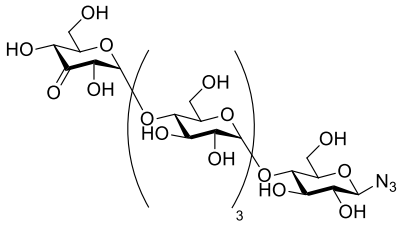
β-D-maltotriosyl azide **4** (190 mg, 0.360 mmol, 1 eq) was dissolved in DMSO (2.4 ml, 0.3 M), before benzoquinone (117 mg, 1.080 mmol, 3 eq) and [(2,9-dimethyl-1,10phenanthroline)-Pd(µ-OAc)]$_2$(OTf)$_2$ (28 mg, 27 µmol, 7.5 mol%) were added. The reaction was stirred at room temperature till complete consumption of starting material (indicated by TLC (eluent: 15% H_2O/CH_3CN). Upon completion the reaction mixture was diluted with H_2O (10 ml) and the resulting aqueous solution was purified by charcoal column chromatography (20% EtOH/H_2O eluted the desired product). The product was freeze dried to yield 121 mg (0.23 mmol, 60%) as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 5.60 (d, *J* = 4.5 Hz, 1H), 5.16 (d, *J* = 3.8 Hz, 1H), 4.51 (d, *J* = 8.6 Hz, 1H), 4.45 (dd, *J* = 4.4, 1.6 Hz, 1H), 4.26 (dd, *J* = 9.6, 1.6 Hz, 1H), 3.93 – 3.71 (m, 8H), 3.65 – 3.43 (m, 6H), 3.18 (appt, *J* = 8.9 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 207.1, 104.8, 102.6, 91.9, 80.6, 80.5, 78.7, 77.9, 77.8, 76.6, 74.6, 74.3, 73.7, 73.3, 73.0, 62.5, 62.0, 61.9 HRMS (ESI) calculated for $C_{18}H_{29}O_{15}N_3Na$ ([M+Na]$^+$): 550.149, found: 550.148 IR νmax/cm^{-1}: 3338 (OH), 2925 (C-H), 2118 (N_3), 1737 (C=O), 1025 (C-O), $[\alpha]_D^{20}$ = + 46.6 (c 1.00, H_2O)

β-D-3-ketomaltoteraosyl azide (13)



β-D-maltotetraosyl azide **5** (55 mg, 0.08 mmol, 1 eq) was dissolved in DMSO (530 µl, 0.15 M), before benzoquinone (26 mg, 0.240 mmol, 3 eq) and [(2,9-dimethyl-1,10-phenanthroline)-Pd(µ-OAc)]$_2$(OTf)$_2$ (12 mg, 12 µmol, 15 mol%) were added. The reaction was stirred at room temperature till complete consumption of starting material (indicated by TLC (eluent: 20% H_2O/CH_3CN)). Upon completion, the reaction mixture was diluted with H_2O (15 ml) and the resulting aqueous solution was purified by charcoal column chromatography (2.5% ^{t}BuOH/H_2O eluted the desired product). The product was freeze-dried to yield an off white solid, containing traces of hydroquinone. Hydroquinone was removed by washing the product in water with diethyl ether to yield 21 mg (0.03 mmol, 38%) of an off-white solid. ^{1}H NMR (400 MHz, D_2O) δ 5.68 (d, *J* = 4.6 Hz, 1H), 5.27 (d, *J* = 4.0 Hz, 1H), 5.24 (d, *J* = 4.1 Hz, 1H), 4.62 (d, *J* = 9.1 Hz, 1H), 4.52 (dd, *J* = 4.6, 1.5 Hz, 1H), 4.32 (dd, *J* = 9.6, 1.5 Hz, 1H), 3.85 – 3.50 (m, 18H), 3.48 (dd, *J* = 9.8, 4.0 Hz, 2H), 3.17 (appt, *J* = 9.0 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 207.2, 102.1, 99.5, 99.3, 89.8, 76.7, 76.6, 76.4, 76.2, 76.1, 75.6, 74.6, 73.2, 73.0, 72.6, 71.5, 71.4, 71.4, 71.1, 70.8, 60.4, 60.4, 60.3, 60.1 HRMS (ESI) calculated for $C_{24}H_{39}O_{20}N_3Na$ ([M+Na]$^+$): 712.202, found: 712.201 IR νmax/cm^{-1}: 3340 (OH), 2932 (C-H), 2121 (N_3), 1738 (C=O), 1027 (C-O), $[\alpha]_D^{20}$= + 102.6 (c 1.00, H_2O)

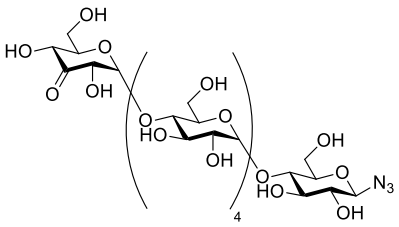
β-D-3-ketomaltopentaosyl azide (14)



β-D-maltopentaosyl azide **6** (58 mg, 0.068 mmol, 1 eq) was dissolved in DMSO (450 µl, 0.15 M), before benzoquinone (22 mg, 0.20 mmol, 3 eq) and [(2,9-dimethyl-1,10-phenanthroline)-Pd(µ-OAc)]$_2$(OTf)$_2$ (6.8 mg, 11 µmol, 15 mol%,) were added. The reaction was stirred at room temperature till complete consumption of starting material (indicated by TLC (eluent: 25% H_2O/CH_3CN)). Upon completion, the reaction mixture was diluted with H_2O (15 ml) and the resulting aqueous solution was purified by charcoal column chromatography (3.0% ^{t}BuOH/H_2O eluted the desired product). The product was freeze-dried to yield an off white solid, containing traces of hydroquinone. Hydroquinone was removed by washing the product in water with diethyl ether to yield 17 mg (0.02 mmol, 30%) of a white solid. ^{1}H NMR (400 MHz, D_2O) δ 5.83 (d, *J* = 4.7 Hz, 1H), 5.44 – 5.35 (m, 3H), 4.76 (d, *J* = 8.4 Hz, 1H). 4.66 (d, *J* = 4.5 Hz, 1H), 4.46 (d, *J* = 9.6 Hz, 1H), 4.02 – 3.74 (m, 18H), 3.74 – 3.58 (m, 7H), 3.31 (appt, *J* = 9.0 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 207.1, 102.0, 99.5, 99.4, 99.3, 89.8, 76.7,

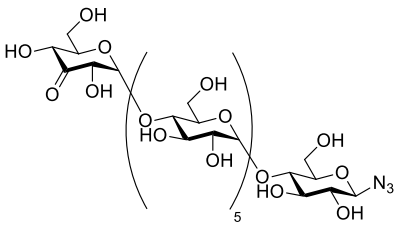
76.7, 76.6, 76.5, 76.3, 76.1, 76.0, 75.6, 74.5, 73.2, 73.1, 72.9, 72.5, 71.5, 71.4, 71.3, 71.0, 71.0, 70.8, 60.3, 60.3, 60.2, 60.2, 60.1. HRMS (ESI) calculated for $C_{30}H_{49}O_{25}N_3Na$ ([M+Na]$^+$): 874.255, found: 874.253 IR νmax/cm^{-1}: 3339 (OH), 2927 (C-H), 2121 (N_3), 1737 (C=O), 1027 (C-O), $[\alpha]_D^{20}$= + 105.6 (c 1.00, H_2O)

β-D-3-ketomaltohexaosyl azide (15)



β-D-maltohexaosyl azide **7** (80 mg, 0.079 mmol, 1 eq) was dissolved in DMSO (530 µl, 0.15 M), before benzoquinone (26 mg, 0.240 mmol, 3 eq) and [(2,9 dimethyl-1,10-phenanthroline)-Pd(µ-OAc)]$_2$(OTf)$_2$ (13 mg, 12 µmol, 15 mol%,) were added. The reaction was stirred at room temperature till complete consumption of starting material (indicated by TLC (eluent: 25 % H_2O/CH_3CN)). Upon completion, the reaction mixture was diluted with H_2O (15 ml) and the resulting aqueous solution was purified by charcoal column chromatography (3.75 % ^{t}BuOH/H_2O eluted the desired product). The product was freeze dried to yield an off white solid, containing traces of hydroquinone. Hydroquinone was removed by washing the product in water with diethyl ether to yield 24 mg (0.024 mmol, 30%) of a white solid. ^{1}H NMR (400 MHz, D_2O) δ 5.60 (d, *J* = 4.6 Hz, 1H), 5.23 – 5.14 (m, 4H), 4.54 (d, *J* = 9.1 Hz, 1H), 4.44 (dd, *J* = 4.7, 1.5 Hz, 1H), 4.24 (dd, *J* = 9.6, 1.6 Hz, 1H), 3.78 – 3.53 (m, 22H), 3.52 – 3.38 (m, 10H), 3.09 (appt, *J* = 9.0 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 207.1, 102.0, 99.5, 99.4, 99.4, 99.3, 89.8, 76.7, 76.7, 76.7, 76.6, 76.5, 76.3, 76.2, 76.1, 76.1, 76.0, 75.6, 74.5, 73.1, 73.1, 72.9, 72.5, 71.4, 71.4, 71.3, 71.0, 71.0, 70.7, 69.1, 60.3, 60.3, 60.2, 60.2, 60.2, 60.0 HRMS (ESI) calculated for $C_{36}H_{59}O_{30}N_3Na$ ([M+Na]$^+$): 1036.31, found: 1036.31 IR νmax/cm^{-1}: 3340 (OH), 2929 (C-H), 2123 (N_3), 1739 (C=O), 1026 (C-O), $[\alpha]_D^{20}$ = + 122.4 (c 1.00, H_2O)

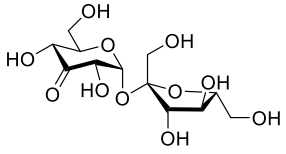
β-D-3-ketomaltoheptaosyl azide (16)



β-D-maltoheptaosyl azide **8** (62 mg, 52.6 µmol, 1 eq) was dissolved in DMSO (350 µl, 0.15 M), before benzoquinone (17 mg, 158 µmol, 3 eq) and [(2,9-dimethyl-1,10-phenanthroline)-Pd(µ-OAc)]$_2$(OTf)$_2$ (8 mg, 8 µmol, 15 mol%,) were added. The reaction was stirred at room temperature till complete consumption of starting material (indicated by TLC (eluent: 40% H_2O/CH_3CN)). Upon completion, the reaction mixture was diluted with H_2O (5 ml) and the resulting aqueous solution was purified by charcoal column chromatography (3.5 % ^{t}BuOH/H_2O eluted the desired product). The product

was freeze-dried to yield an off white solid, containing traces of hydroquinone. Hydroquinone was removed by washing the product in water with diethyl ether to yield 29 mg (24.5 μmol, 47%) of an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 5.69 (d, *J* = 4.6 Hz, 1H), 5.32 – 5.22 (m, 5H), 4.63 (d, *J* = 8.1 Hz, 1H), 4.53 (dd, *J* = 4.6, 1.6 Hz, 1H), 4.32 (d, *J* = 9.8 Hz, 1H), 3.89 – 3.57 (m, 36H), 3.52 (m, 16H), 3.18 (appt, *J* = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 207.2, 102.1, 99.5, 99.5, 99.5, 99.3, 89.8, 76.7, 76.5, 76.4, 76.2, 76.1, 75.6, 74.8, 74.6, 73.2, 73.2, 73.0, 72.6, 71.5, 71.5, 71.4, 71.1, 71.1, 70.8, 60.4, 60.4, 60.3, 60.3, 60.1 (12 signals are missing due to severe overlap). HRMS (ESI) calculated for $C_{42}H_{69}O_{35}N_3Na$ ([M+Na]$^+$): 1198.36, found: 1198.36 IR νmax/cm^{-1}: 3343 (OH), 2924 (C-H), 2119 (N_3), 1738 (C=O), 1025 (C-O), $[\alpha]_D^{20}$ = + 120.2 (c 1.00, H_2O)

3-keto-sucrose (18)



Sucrose (62 mg, 0,18 mmol, 1 eq) and benzoquinone (58 mg, 0.54 mmol, 3 eq) were dissolved in DMSO-d_6 (600 μl, 0.3 M) and transferred to a NMR tube. T1 was determined followed by a start NMR to determine the ratio of DMSO : Starting material. [(Neocuproine)PdOAc]$_2$OTf$_2$ (4.7 mg, 4.5 μmol, 2.5 mol%) was added to the NMR tube, mixed and reacted for 1h. Selectivity towards the desired product: 50 % ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.60 (d, *J* = 4.5 Hz, 1H, H1), 4.27 (d, *J* = 4.4 Hz, 1H, H2), 4.14 (d, *J* = 9.7 Hz, 1H, H4), 3.94 – 3.86 (m, 2H, H3' + H5), 3.75 – 3.61 (m, 3H, H4' + H6), 3.61 – 3.55 (m, 3H, H5' + H6'), 3.50 – 3.41 (m, 1H, H1a'), 3.40 – 3.32 (m, 1H, H1b'). ^{13}C NMR (101 MHz, DMSO-d_6) δ 206.6 (C3), 104.5 (C2'), 94.5 (C1), 82.8 (C5'), 76.2 (C3'), 75.6 (C5), 74.2 (C2), 74.1 (C4'), 71.6 (C4), 62.3 (C6'), 61.8 (C1'), 60.4 (C6).

2.4.5 Bis functionalization of protein

Cu catalysed click reaction on the protein 4-OT

The alkyne bearing protein 4-OT[25] (18 μl of a 0.39 mg/ml solution in 50 mM phosphate buffer, pH 8.0) was glycosylated with compound **12** and **16** (0.5 μl of a 10 mM solution in water), respectively, in the presence of CuSO4 (0.5 μl of a 40 mM solution in water), sodium ascorbate (0.5 μl of a 50 mM solution in water) and the ligand THPTA (0.5 μl of a 4 mM solution in water). The reaction was mixed and left for 16 h at rt. The concentrations of the solutes in the resulting solution (20 μl) were as follows: 4-OT, 50 μM; oligomaltoside, 250 μM; CuSO4, 1 mM; sodium ascorbate, 1.25 mM; THPTA, 100 μM. Subsequently, the samples were separated with Tricine SDS-PAGE[27] on a 16% gel and visualized with Coomassie Brilliant Blue R250 staining (AMRESCO, Solon, OH, USA).

Preconjugation with Biotin followed by click reaction

Compounds **12** and **16** were incubated each with hydrazide modified biotin (glycoside: 10 μl of a 10 mM solution in water and biotin hydrazide: 90 μl of a 55

mM solution in DMSO) after thorough mixing by vortexing, for 24 h at rt, to allow acyl hydrazone formation.The alkyne bearing protein 4-OT[25] (18 μl of a 0.39 mg/ml solution in 50 mM phosphate buffer, pH 8.0) was glycosylated with the biotin bearing compounds **12** and **16** (0.5 μl of a 10 mM solution in water), respectively, using copper catalysed alkyne azide cycloaddition (CuAAC). Triazole formation was conducted in the presence of CuSO4 (0.5 μl of a 40 mM CuSO4 solution in water), sodium ascorbate (0.5 μl of a 50 mM solution in water) and the ligand THPTA (0.5 μl of a 4 mM solution in water). The reaction was mixed and left for 16 h at rt. The concentrations of the solutes in the resulting solution (20 μl) were as follows: 4-OT, 50 μM; oligomaltoside, 250 μM; CuSO4, 1 mM; sodium ascorbate, 1.25 mM; THPTA, 100 μM. The modified proteins were subjected to Tricine SDS-PAGE and subsequently transferred to a PVDF membrane for visualisation via ECL. To this end the protein samples were separated on a 16% tricine gel, then blotted onto the PVDF membrane (GE Healthcare, Wauwatosa, WI, USA) using a Bio-Rad (Hercules, CA, USA) Mini *Trans*-Blot system for wet blotting according to the manufacturer's protocol. Electro blotting was followed by blocking the membrane with 5% non-fat dry milk (Sigma-Aldrich St. Louis, MO, USA) in PBS buffer, and then washing 3X for 10 min each with PBS-T. The membrane was probed with HRP-conjugated Streptavidin (ThermoFisher Scientific, Waltham, MA, USA) in 5% non-fat dry milk in PBS-T buffer (1:10,000) for one hour at room temperature. The chemiluminescence signals were recorded subsequent to washing, 3X for 10 min with PBS-T buffer and 2X for 10 min with PBS buffer, using a Bio-Rad ChemiDoc XRS+ system and Clarity Western ECL Substrate according to the manufacturer's protocol

2.5 References

(1) White, M. *Synlett* **2012**, *1*, 2746–48.

(2) Jeffrey, J. L.; Terrett, J. A.; MacMillan, D. W. C. *Science* **2015**, *349*, 1532–36.

(3) Jäger, M.; Minnaard, A. J. *Chem. Commun.* **2016**, *52*, 656–64.

(4) Jordan, P. A.; Miller, S. J. *Angew. Chem. Int. Ed.* **2012**, *51*, 2907–11.

(5) Bierenstiel, M.; Schlaf, M. *Eur. J. Org. Chem.* **2004**, *2004*, 1474–81.

(6) Muramatsu, W. *Org. Lett.* **2014**, *16*, 4846–49.

(7) Painter, R. M.; Pearson, D. M.; Waymouth, R. M. *Angew. Chem. Int. Ed.* **2010**, *49*, 9456–59.

(8) Wu, Z.; Jäger, M.; Buter, J.; Minnaard, A. J. *Beilstein J. Org. Chem.* **2013**, *9*, 2374–77.

(9) Styslinger, T. J.; Zhang, N.; Bhatt, V. S.; Pettit, N.; Palmer, A. F.; Wang, P. G. *J. Am. Chem. Soc.* **2012**, *134*, 7507–15.

(10) Tanaka, T.; Matsumoto, T.; Noguchi, M.; Kobayashi, A.; Shoda, S. *Chem. Lett.* **2009**, *38*, 458–59.
(11) Tanaka, T.; Huang, W. C.; Noguchi, M.; Kobayashi, A.; Shoda, S. *Tetrahedron Lett.* **2009**, *50*, 2154–57.
(12) Noguchi, M.; Tanaka, T.; Gyakushi, H.; Kobayashi, A.; Shoda, S. *J. Org. Chem.* **2009**, *74*, 2210–12.
(13) Lim, D.; Brimble, M. A.; Kowalczyk, R.; Watson, A. J. A.; Fairbanks, A. J. *Angew. Chem. Int. Ed.* **2014**, *53*, 11907–11.
(14) Tanaka, T.; Nagai, H.; Noguchi, M.; Kobayashi, A.; Shoda, S. *Chem. Commun.* **2009**, *23*, 3378.
(15)
(16) Lindsay, V. N. G.; Lindsay; G., V. N. In *Encyclopedia of Reagents for Organic Synthesis*; John Wiley & Sons, Ltd: Chichester, UK, 2012.
(17) Whistler, R. L.; Durso, D. F. *J. Am. Chem. Soc.* **1950**, *72*, 677–79.
(18) Bock, K.; Pedersen, C.; Pedersen, H. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 193–225.
(19) Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.
(20) Sedmera, P.; Halada, P.; Kubátová, E.; Haltrich, D.; Přikrylová, V.; Volc, J. *J. Mol. Catal. B Enzym.* **2006**, *41*, 32–42.
(21) Pertici, F.; de Mol, N. J.; Kemmink, J.; Pieters, R. J. *Chem. Eur. J.* **2013**, *19*, 16923–27.
(22) Herbert, J.-M.; Petitou, M.; Hérault, J.-P.; Bernat, A.; Driguez, P.-A.; Duchaussoy, P.; Lormeau, J.-C. *Nature* **1999**, *398*, 417–22.
(23) Fyrner, T.; Magnusson, K.; Nilsson, K. P. R.; Hammarström, P.; Aili, D.; Konradsson, P. *Bioconjug. Chem.* **2012**, *23*, 1333–40.
(24) Schneider, M. F.; Mathe, G.; Tanaka, M.; Schmidt, R. R. *J. Phys. Chem. B* **2001**, *105*, 5178–85.
(25) Ourailidou, M. E.; Dockerty, P.; Witte, M.; Poelarends, G. J.; Dekker, F. J. *Org. Biomol. Chem.* **2015**, *13*, 3648–53.
(26) Conley, N. R.; Labios, L. A.; Pearson, D. M.; McCrory, C. C. L.; Waymouth, R. M. *Organometallics* **2007**, *26*, 5447–53.
(27) Schägger, H. *Nat. Protoc.* **2006**, *1*, 16–22.